

Center for Musculoskeletal Research

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Musculoskeletal Cell Core

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Osteoblasts Differentiations

Material:

- BGlycerophosphate (bGPO) (Sigma G9422-10G)
- L- ascorbic acid (Sigma A4403-100MG)
- □MEM complete medium (□MEM +10% Fetal Bovine Serum+ 1% Pen-strep or Antimycotic-Antibiotic)

Primary calvaria osteoblasts are isolated following “Primary Calvaria Protocol”

Once confluent cells are trypsinized and re-plated (P1) in BD Biocoat-collagen coated plates at 50-100,000 cells/ml in 12, 6 or 10 cm dishes depending on downstream applications.

For differentiation (time course),
cells can be analyzed at early (3-7 days), 14 and late (21-35 days).

For mineralization assay medium needs to be supplemented with 10mM □Glycerophosphate (bGPO) and 50 µg/ml of ascorbic acid (□MEM does contain AA so this one can be potentially be omitted)

Prepare 1000X stock of both AA and bGPO and store them at -20C protected from light. Add them fresh every time (add to □MEM complete medium and filter)

Change medium three times/week (Mon-Wed-Fri)

To collagen coat plate:

Material:

- Sterile collagen solution (rat tail) stored at 4 C (becton Dickinson Cat# 354236) STERILE
- Sterile 0.02N Acetic acid solution (best if stored at 4 C)
- Pre-chilled pipettes

Dilute sterile collagen Type 1 (Rat Tail) solution with 0.02 M Acetic Acid to a final concentration of 0.15mg/ml
The concentration of the stock is written on the bottle and it varies from batch to batch

Use a chilled pipette so the collagen does not stick too much

**NB: the collagen solution can be re-used few times (5-6) and should be kept in the refrigerator

Coat plate for 1hr at room temperature. Remove and save collagens solution

Before using the plate rinse twice with sterile PBS and once with medium. If medium turns yellow then some acetic acid is still in the plate and it is best to wash one more time

If plates are going to be stored (@ 4C) for later use. Dry the plates for 1 hr with lid off before storing them at 4C
Collagen coated plates can also be purchase from BD Biocoat (Corning)

